

RAS oncogene

The Ras Oncogene

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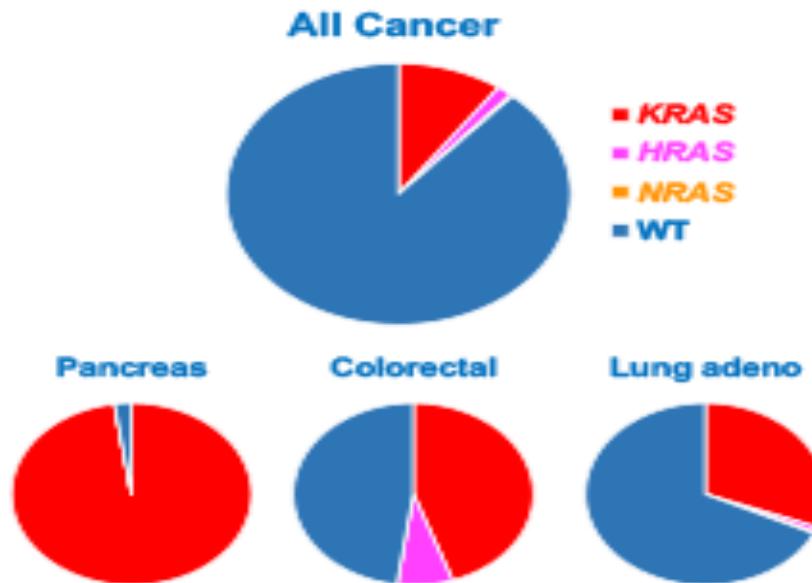
TRACO Lecture
Oct 28, 2019



Research goals

Our research goals

- Understand mechanism of KRAS-driven oncogenesis
- Identify better therapeutic strategies for KRAS mutant tumors



Clinical Challenge

- ~ 200,000 new patients / year
- No effective targeted therapies

Outline

Lecture Outline

1. Discovery of the Ras oncogene
2. Ras signaling & oncogenesis
3. Targeting Ras and its signaling network
4. Synthetic lethal partners of the KRAS oncogene
5. Identify optimal target combinations for KRAS mutant cancer cells
6. Concluding thoughts

RAS oncogene

Discovery of the Ras Oncogene in Murine Tumor Viruses

1964

Harvey (Nature, 1964) An Unidentified virus which causes the rapid production of tumors in mice

1967

Kirsten & Mayer (J. NCI, 1967) Morphologic response to a murine erythroblastosis virus



Molecular characterization

Molecular Characterization of the Viral Ras Protein

1979

Shih ... Scolnick (Virology, 1979) Identification of a sarcoma virus-coded phosphoprotein in nonproducer cells transformed by Kirsten or Harvey murine sarcoma virus
Scolnick... Shih (PNAS, 1979) Guanine nucleotide-binding activity as an assay for *src* protein of rat-derived murine sarcoma viruses

1980

Willingham ... Scolnick (Cell, 1980) Localization of the *src* gene product of the Harvey strain of MSV to plasma membrane of transformed cells by electron microscopic immunocytochemistry



Mammalian counterpart

Viral Ras Gene Has Mammalian Counterpart

1981

Ellis ... Scolnick (Nature, 1981) The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes

1982

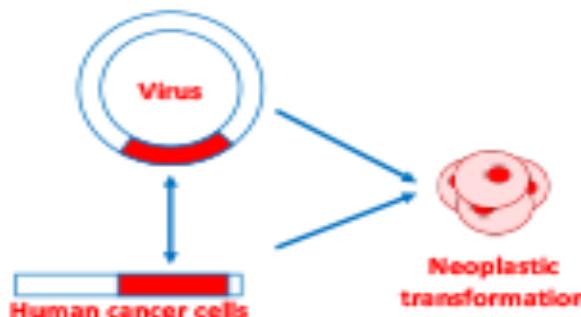
Chang ... Lowy (Nature, 1982) Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus

Santos ... Barbacid (Nature, 1982) T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes

Parada ... Weinberg (Nature, 1982) Human EJ bladder carcinoma oncogene is homologous of Harvey sarcoma virus *ras* gene

Goldfarb ... Wigler (Nature, 1982) Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells.

Der ... Cooper (PNAS, 1982) Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses



Point mutations

Human Ras Oncogene Has a Point Mutation

1982

Tabin ... Weinberg (Nature, 1982) Mechanism of activation of a human oncogene

Reddy ... Barbacid (Nature, 1982) A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene

Taparowsky ... Wigler (Nature, 1982) Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change.

Capon ... Goeddel (Nature, 1982) Activation of Ki-ras2 gene in human colon and lung carcinomas by two different point mutations



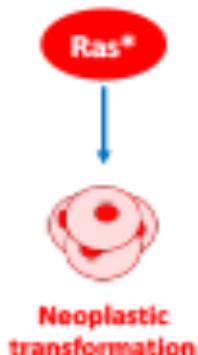
Transforming oncogene

Human Ras Oncogene Encodes a Transforming Oncoprotein

1984

1985

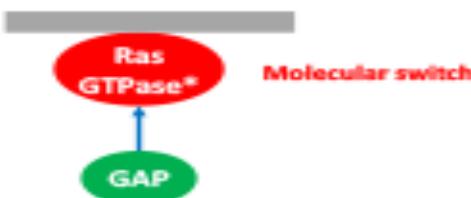
Stacey ... Kung (Nature, 1984) Transformation of NIH 3T3 cells by microinjection of *Ha-ras* p21 protein
Feramisco ... Sweet (Cell, 1984) Microinjection of the oncogene form of the human H-ras (T-24) protein results in rapid proliferation of quiescent cells
Mulcahy ... Stacey (Nature, 1985) Requirement for *ras* proto-oncogene function during serum-stimulated growth of NIH 3T3 cells



Biochemical properties

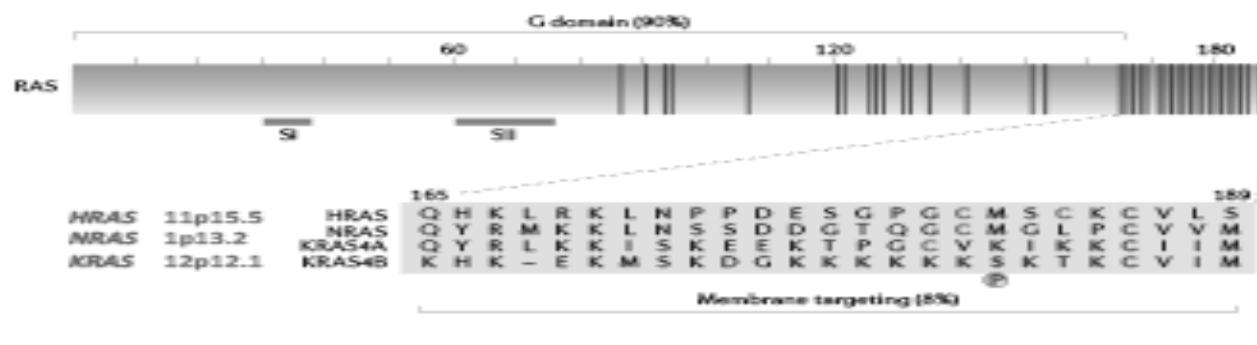
Biochemical Properties of the Ras Protein

1984	Willumsen & Lowy (<i>Nature</i> , 1984) The p21 ras C-terminus is required for transformation and membrane association
1984	Sweet ... Lowy (<i>Nature</i> , 1984) The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity
1985	Gibbs ... Scolnick (<i>PNAS</i> , 1984) Intrinsic GTPase activity distinguishes normal and oncogenic ras p21 molecules
1985	Hanne ... Kung (<i>PNAS</i> , 1985) <i>Ha-ras</i> proteins exhibit GTPase activity: point mutations that activate <i>Ha-ras</i> gene products result in decreased GTPase activity
1987	Trahey ... McCormick (<i>Science</i> , 1987) A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants



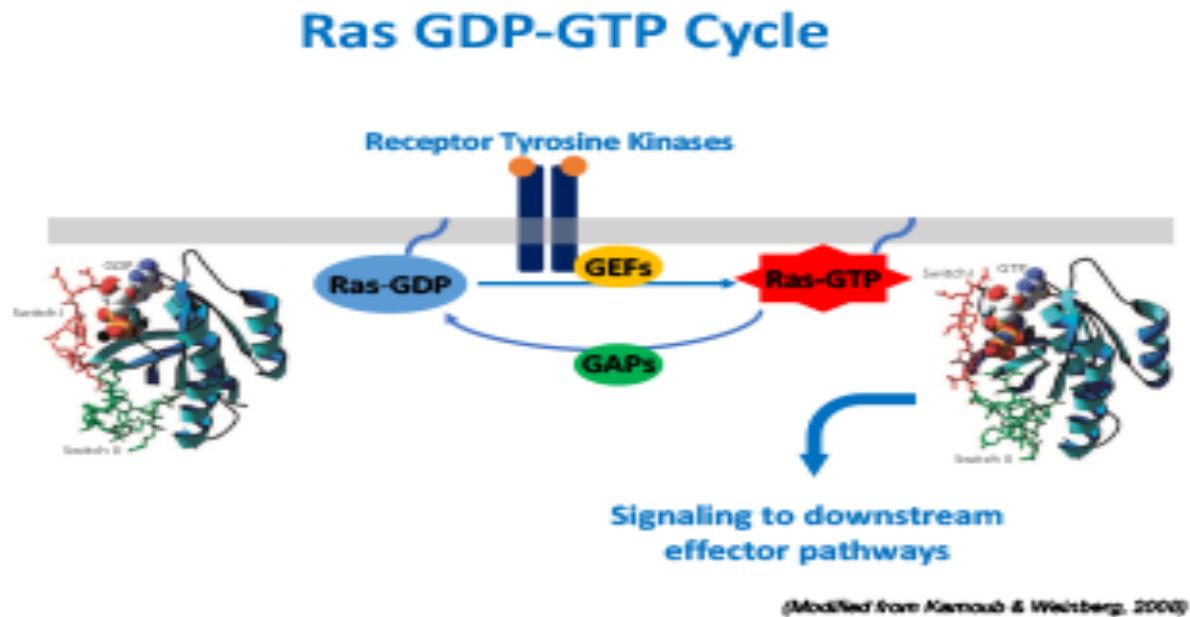
Ras family

The Ras Family of Small GTPases

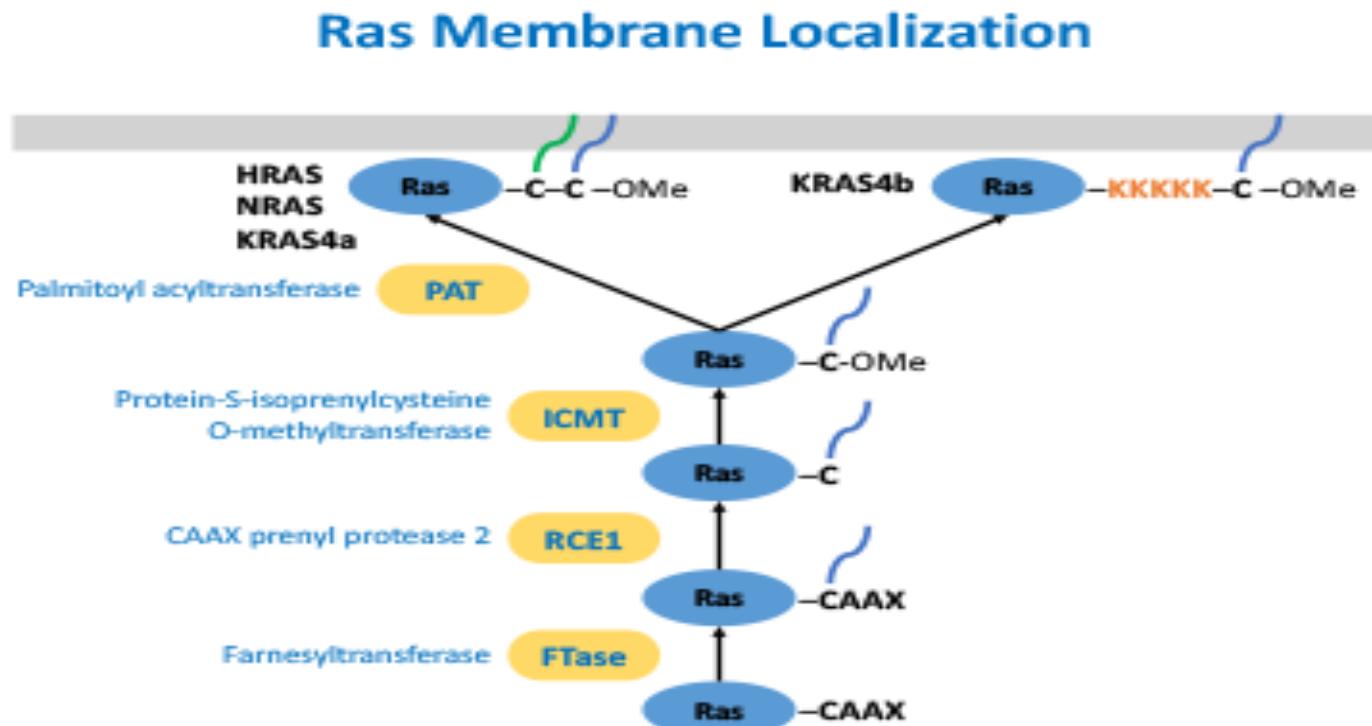


(Cox et al, Nature Review Drug Discovery 2014)

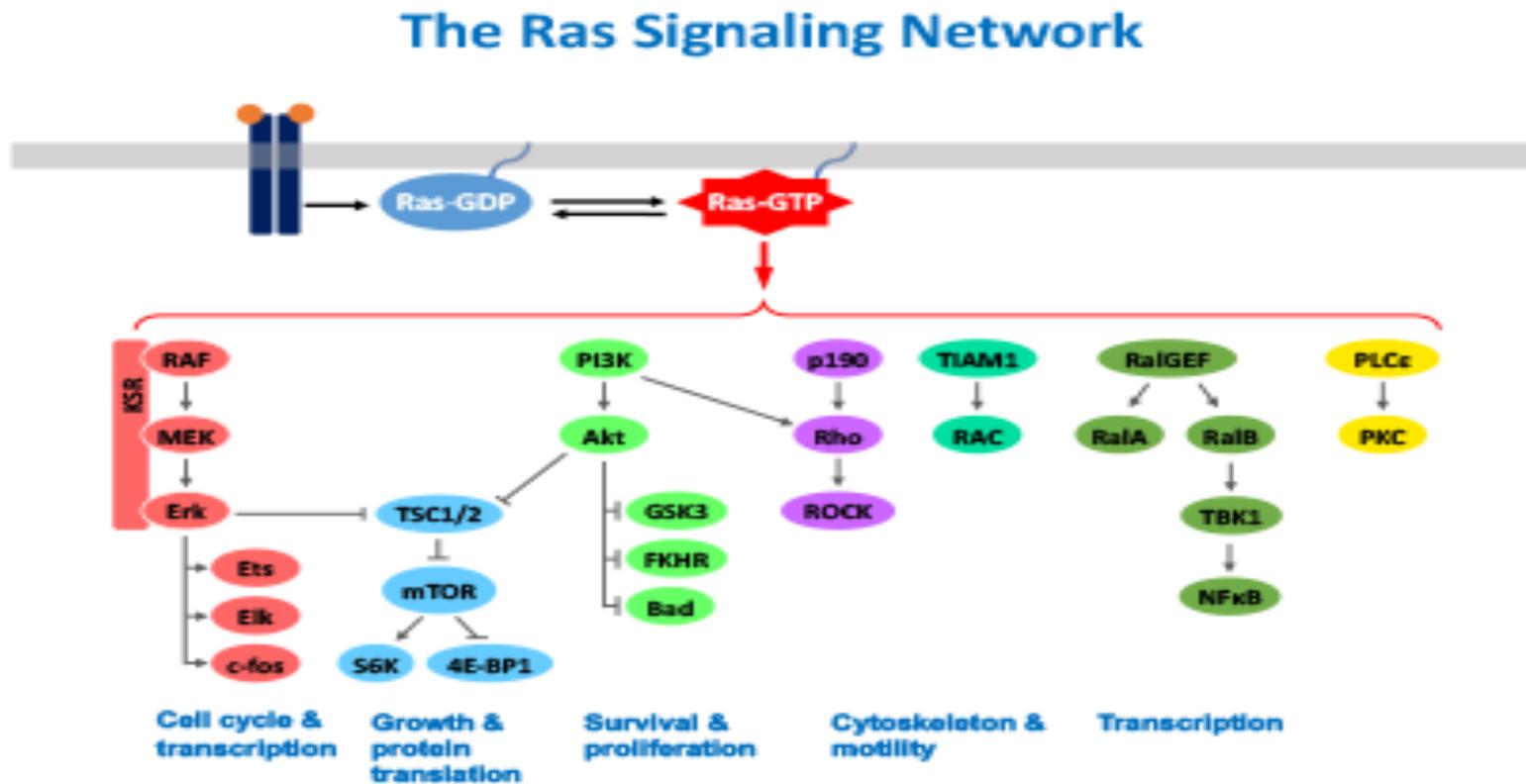
Ras GDP-GTP Cycle



Ras membrane localization

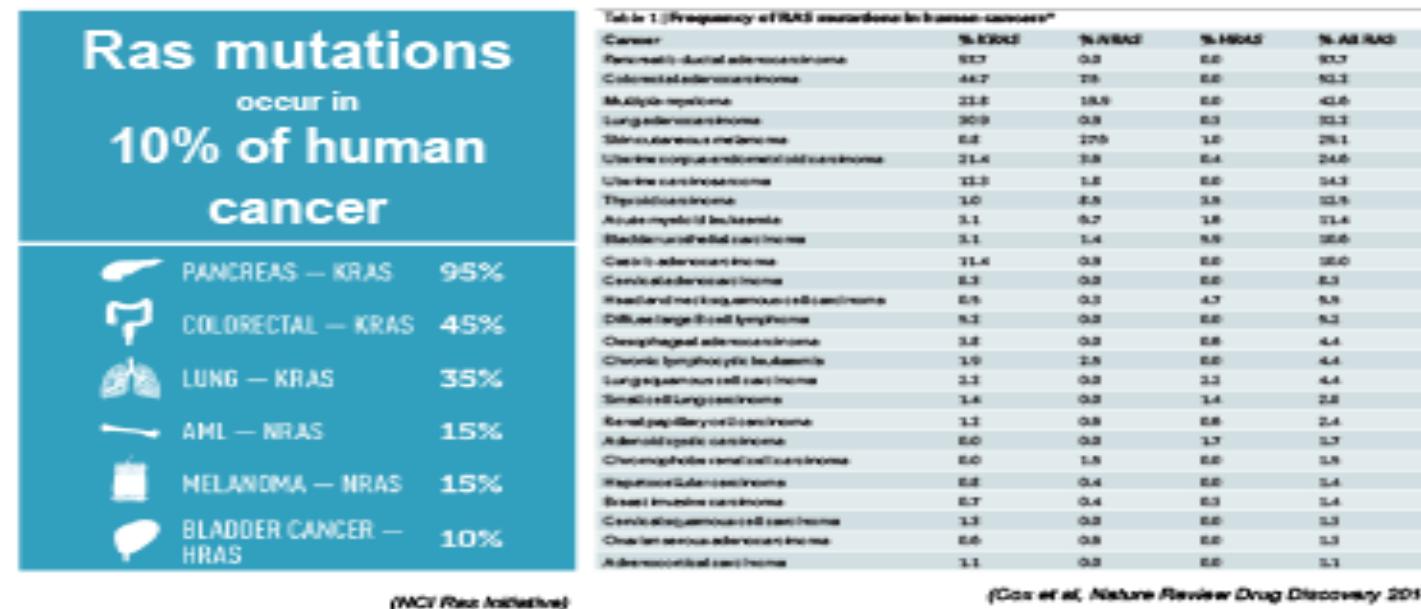


Ras signaling network

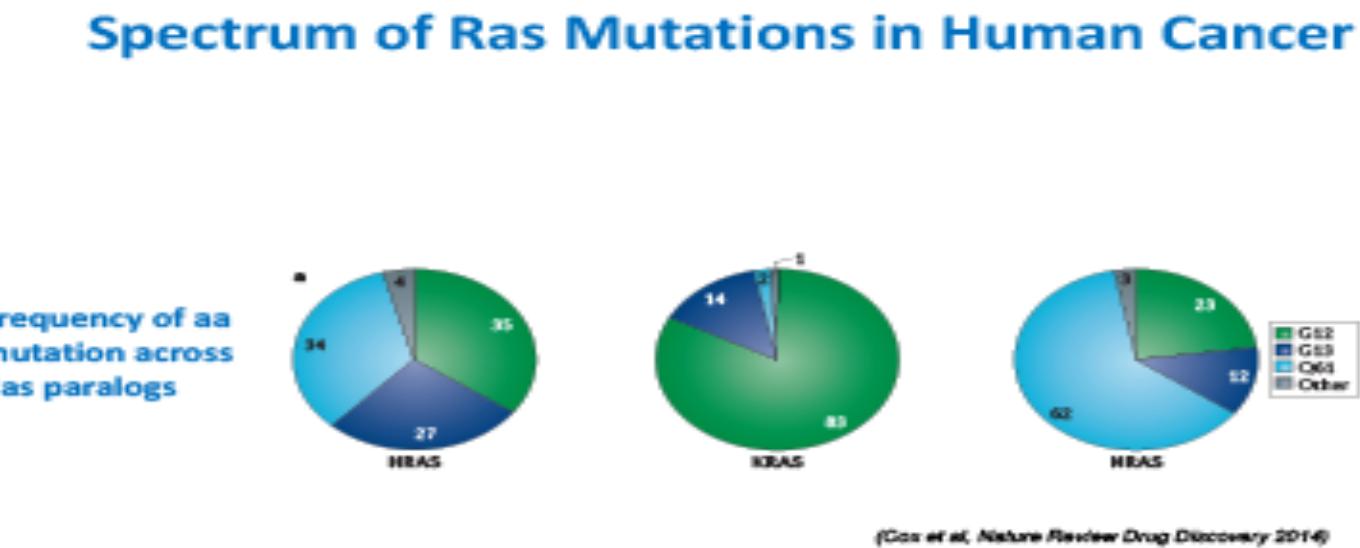


Ras mutations

Incidence of Ras mutations in human cancer

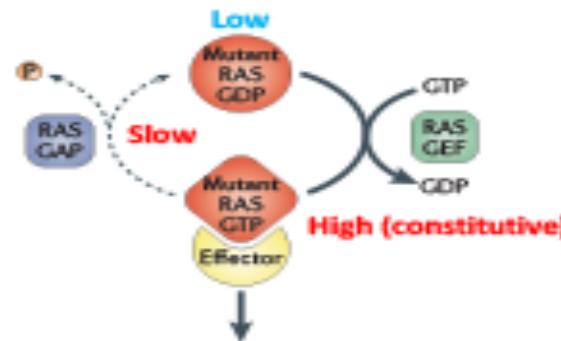
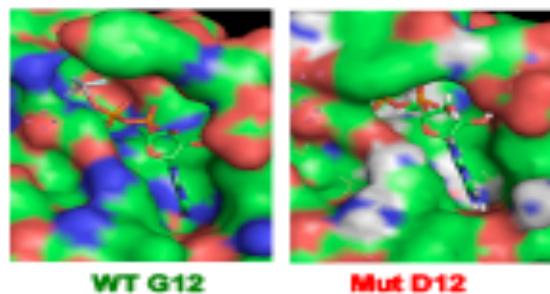
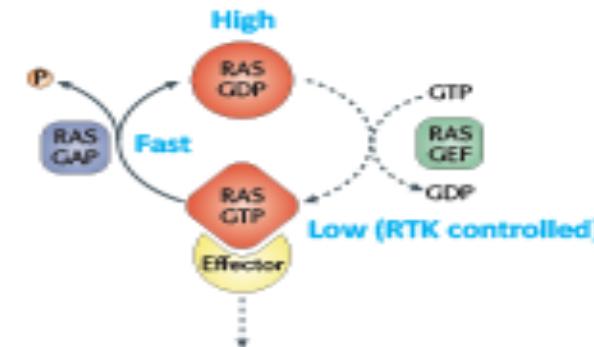
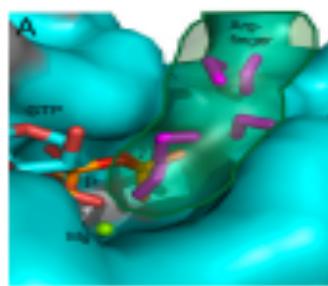


Mutation spectrum



GTP hydrolysis

Oncogenic Ras Mutations Impair GTP Hydrolysis



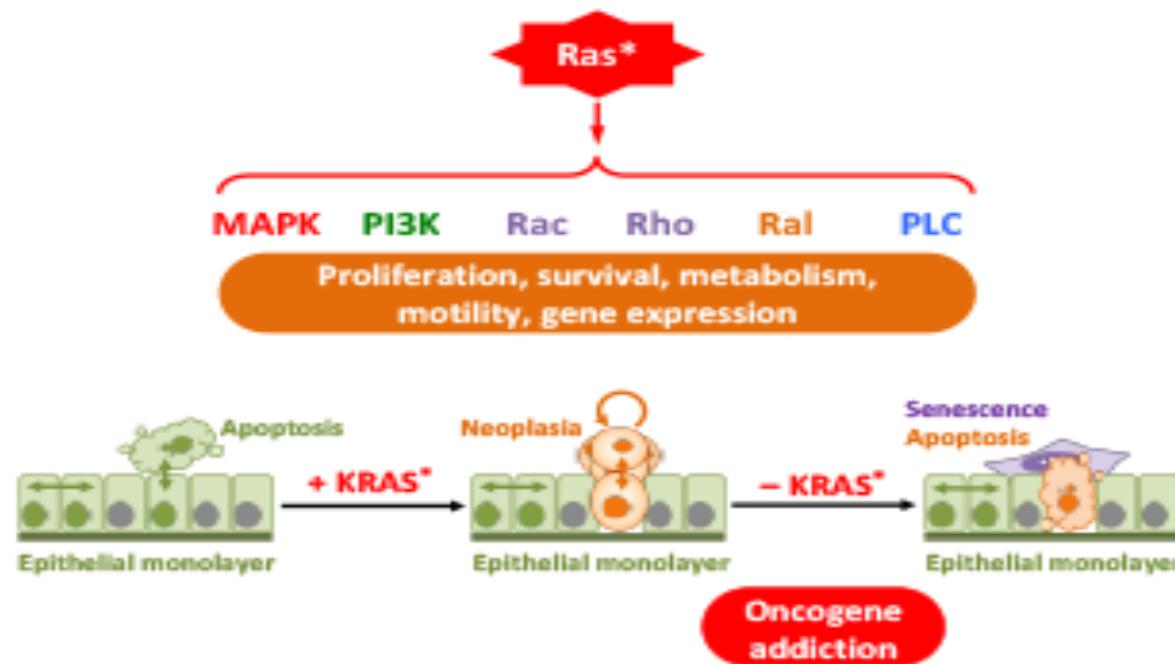
Oncogenic driver

KRAS is a Major Oncogenic Driver in Adenocarcinomas

	Early Neoplasia	Adenoma	Adeno-carcinoma
Lung	KRAS	KRAS TP53	KRAS TP53 LKB1 ***
Pancreas	KRAS	KRAS CDKN2A	KRAS CDKN2A TP53 ***
Colon	APC	APC KRAS	APC KRAS TP53 ***

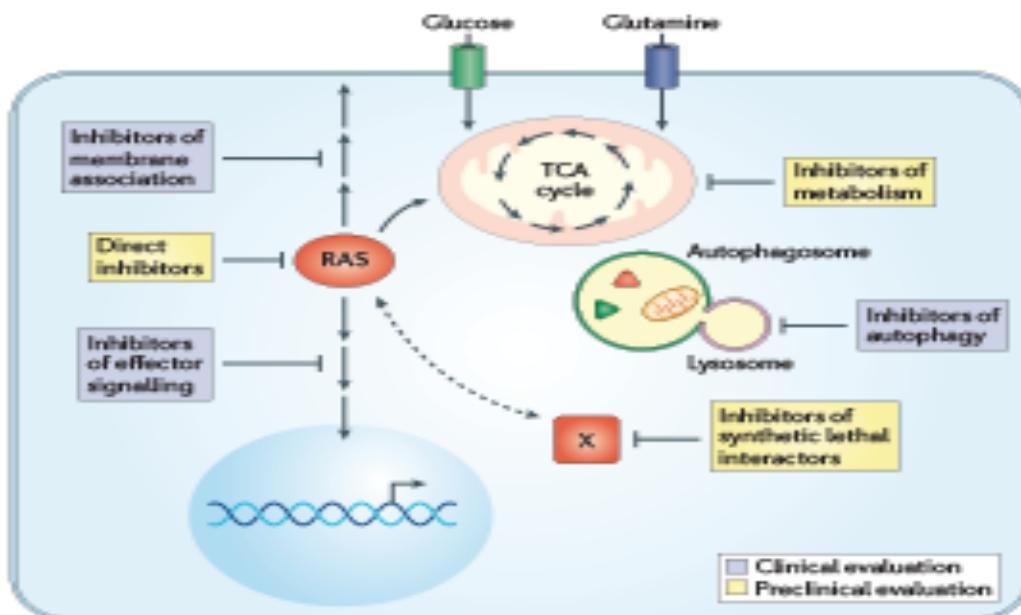
Neoplastic transformation

Oncogenic Ras Signaling Leads to Neoplastic Transformation



Ras signaling

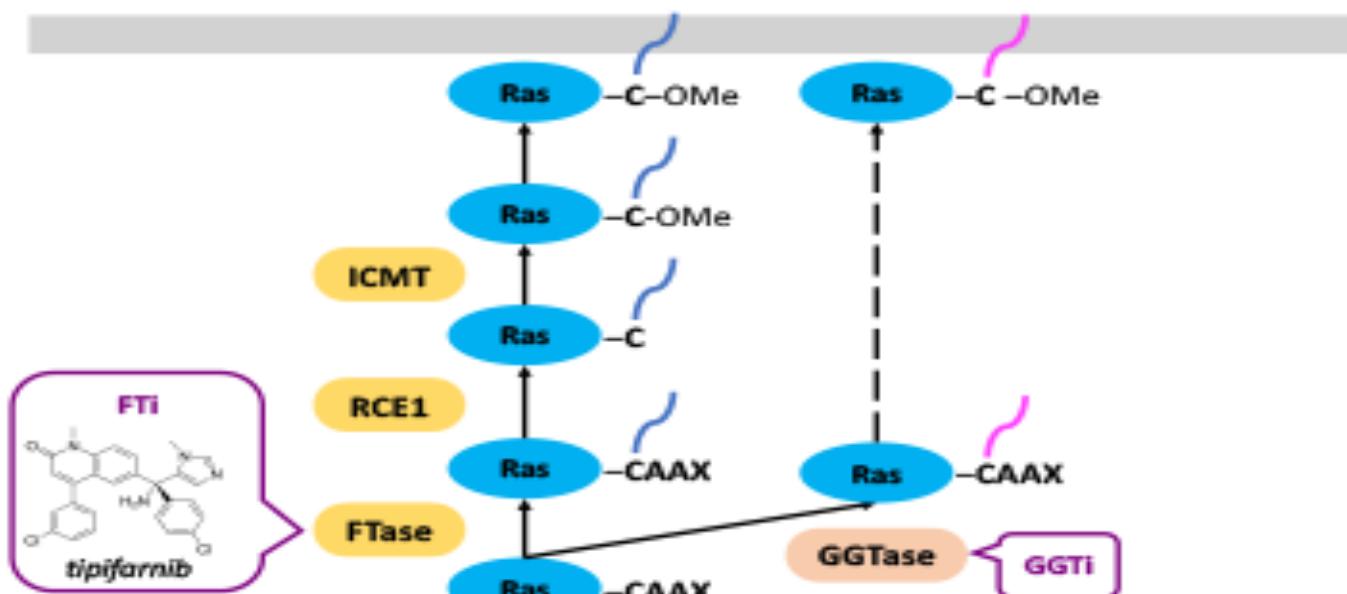
Strategies to Target Oncogenic Ras Signaling



(Cox et al., 2016)

Ras membrane association

Inhibition of Ras Membrane Association



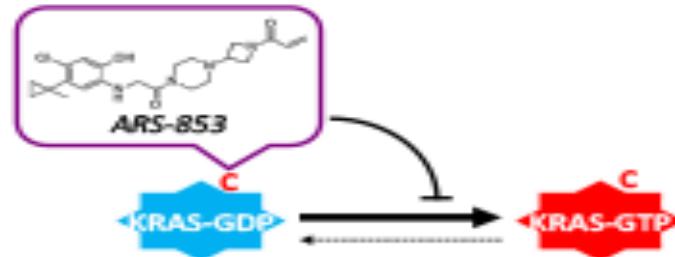
- Alternative membrane localization pathway
- Lack of KRAS selectivity – pathway shared by other small GTPases

Ras inhibition

Direct Inhibition of Ras Oncoprotein Function

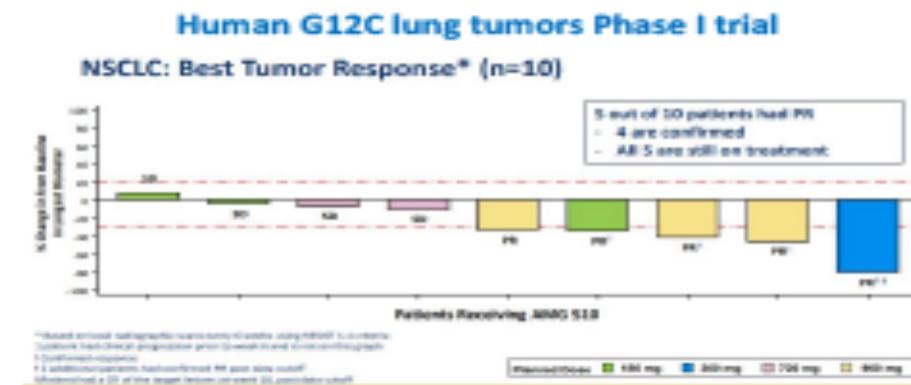
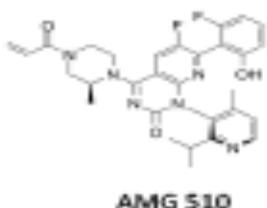
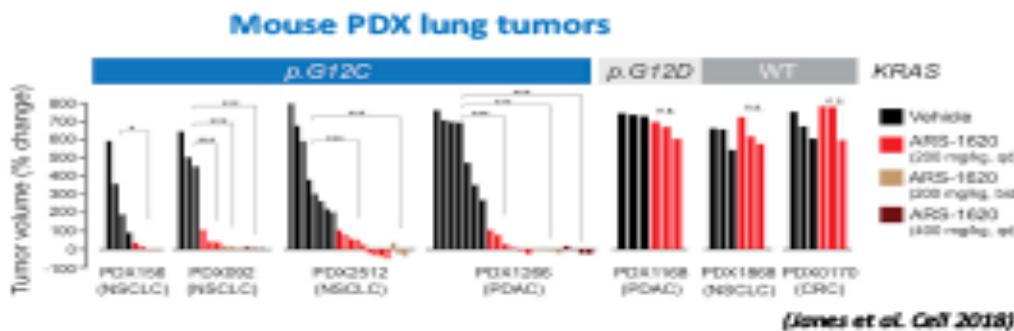
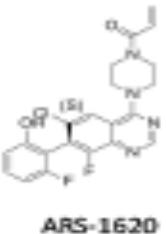


KRAS-G12C covalent inhibitors



Direct inhibition

Direct Inhibition of Ras Oncoprotein Function



(Amgen; ASCO 2019)

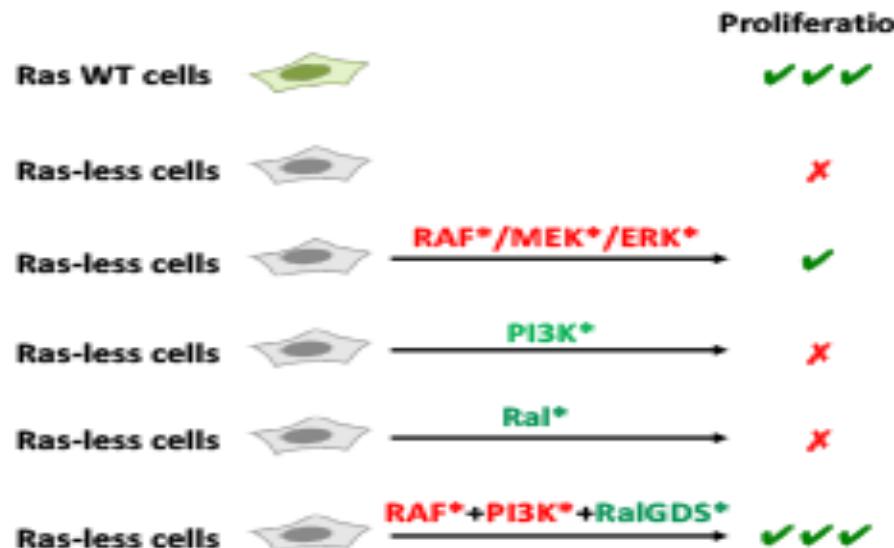
Kinase inhibitors

Kinase Inhibitors Targeting Ras Effectors



MAPK pathway

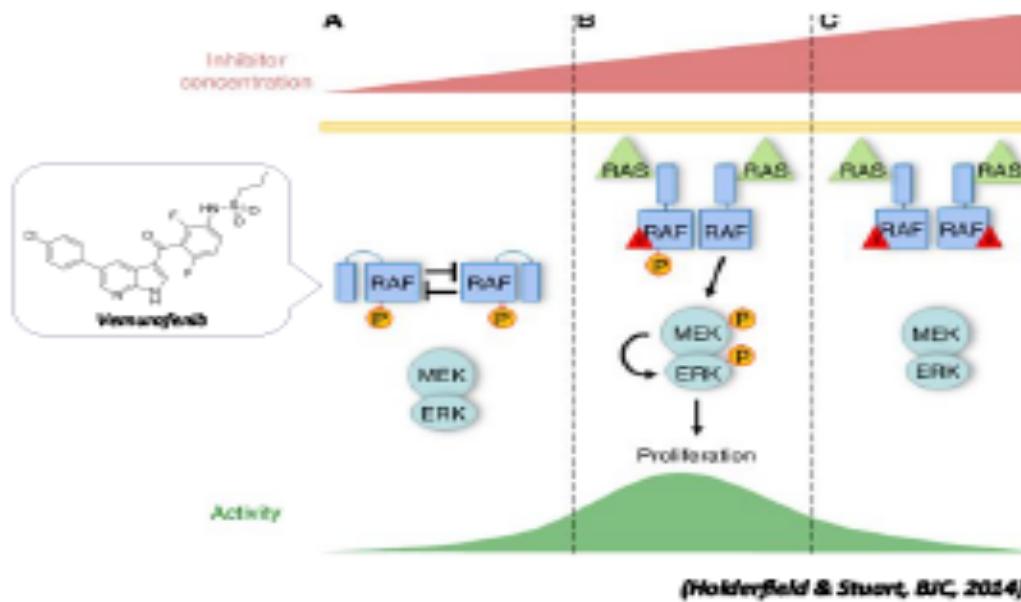
MAPK Pathway is Essential for Ras-Driven Cell Proliferation



(Drost et al., EMBO J., 2010)

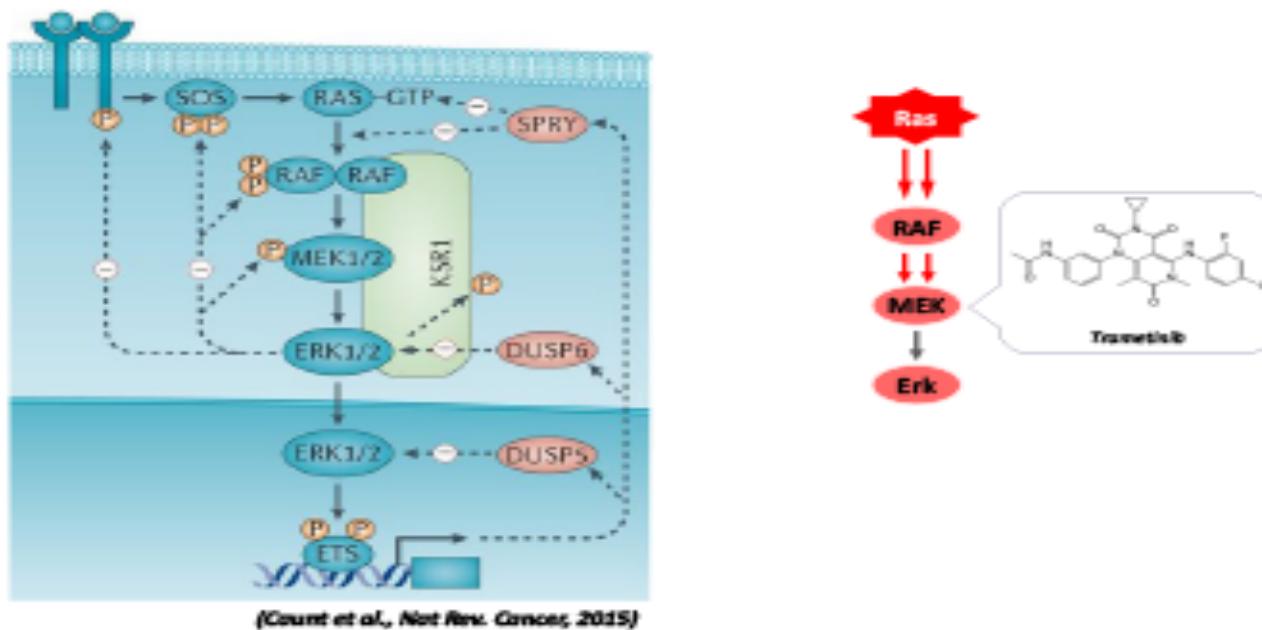
MAPK pathway

RAF Inhibitors Activates MAPK Pathway in Ras-Mutant Cells



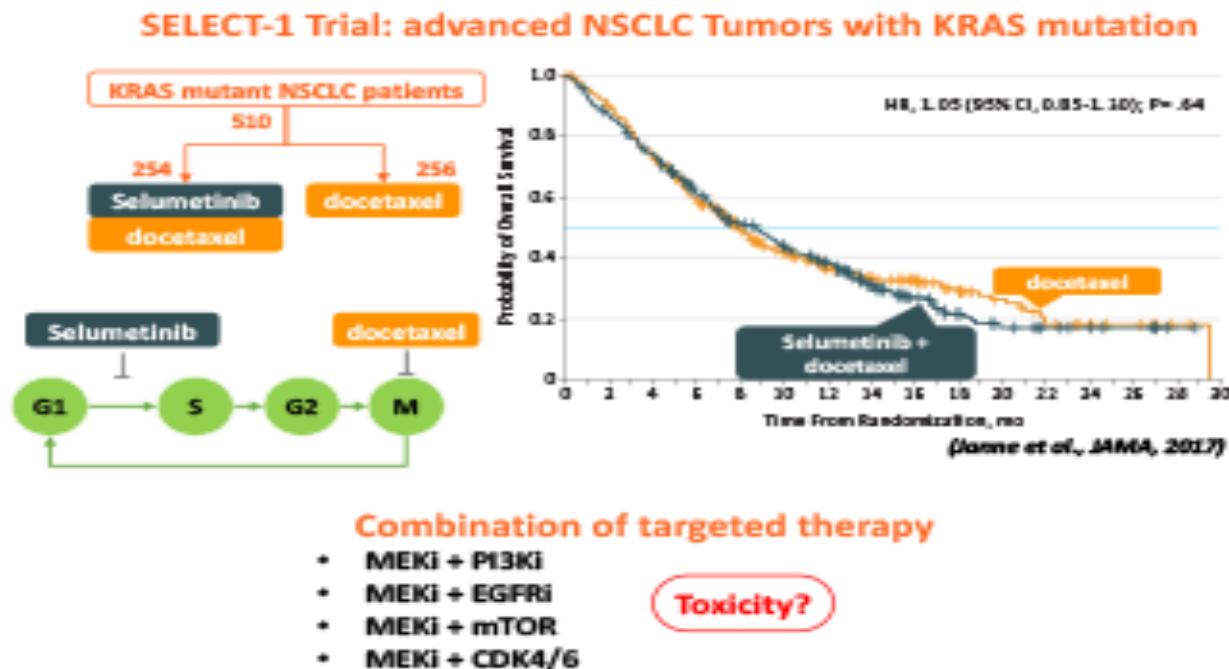
Feedback activation

MEK Inhibitors Leads to Feedback Activation of the MAPK Pathway



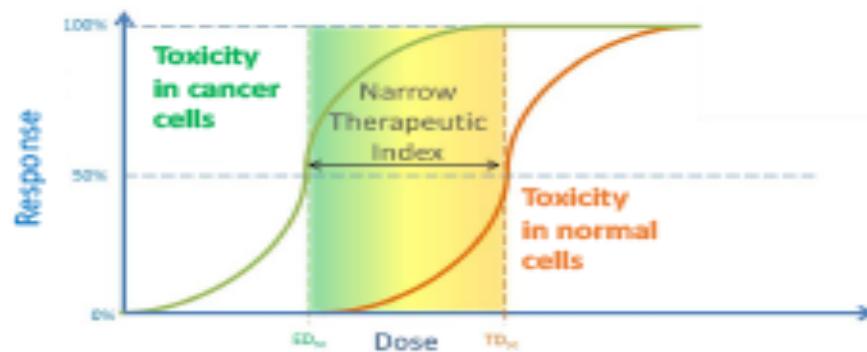
MEKi combinations

Current MEKi Combinations Are Ineffective in KRAS Mutant Cancer



Therapeutic window

Therapeutic Window is Key to Effective Combination Therapy in Cancer



Good combination therapies

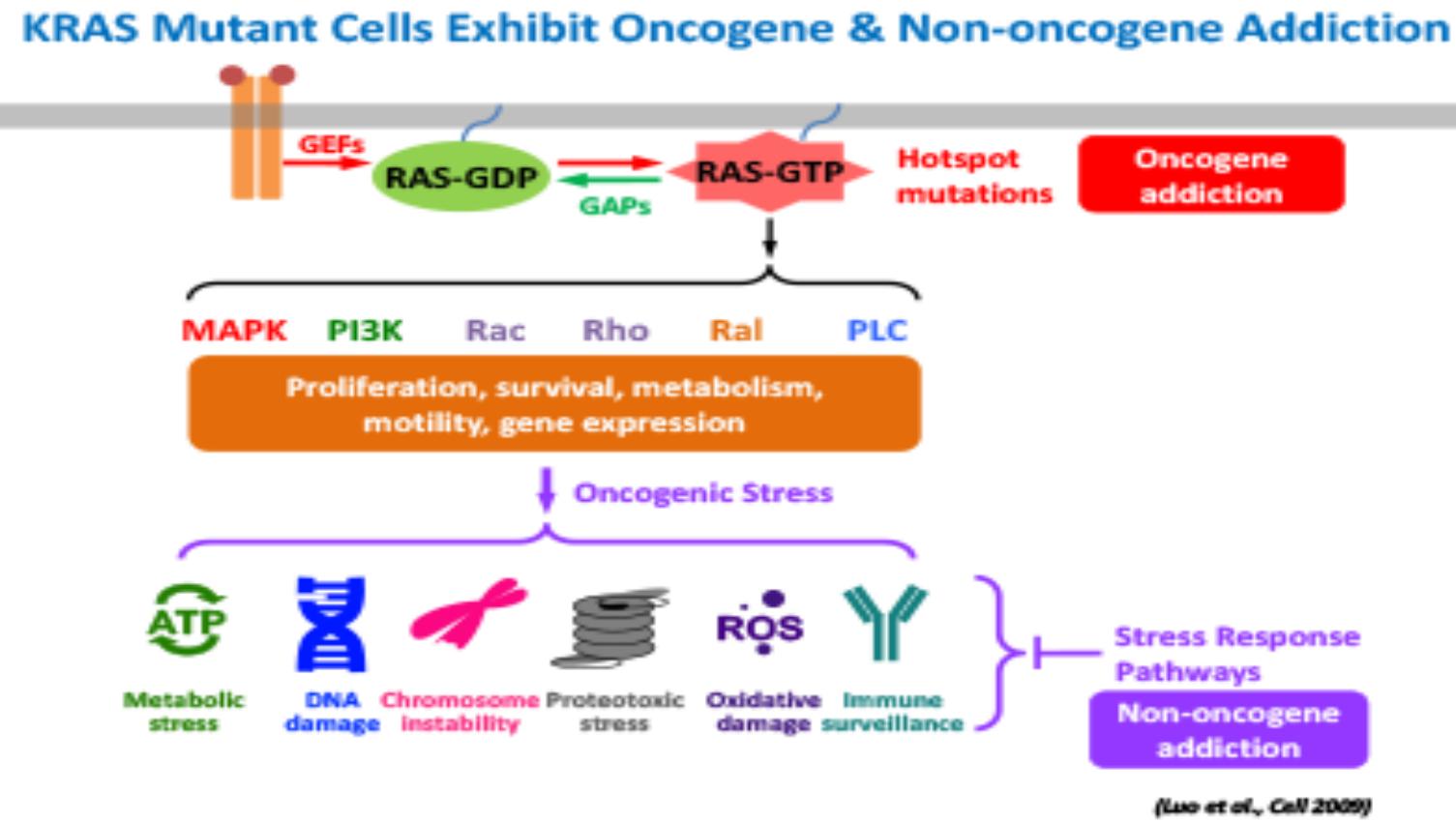
- Confer genotype-specific synergy
- Widens the therapeutic window
- Delays on-set of drug resistance

Synthetic lethality

Synthetic Lethality Reflects Genetic Buffering and Pathway Redundancy

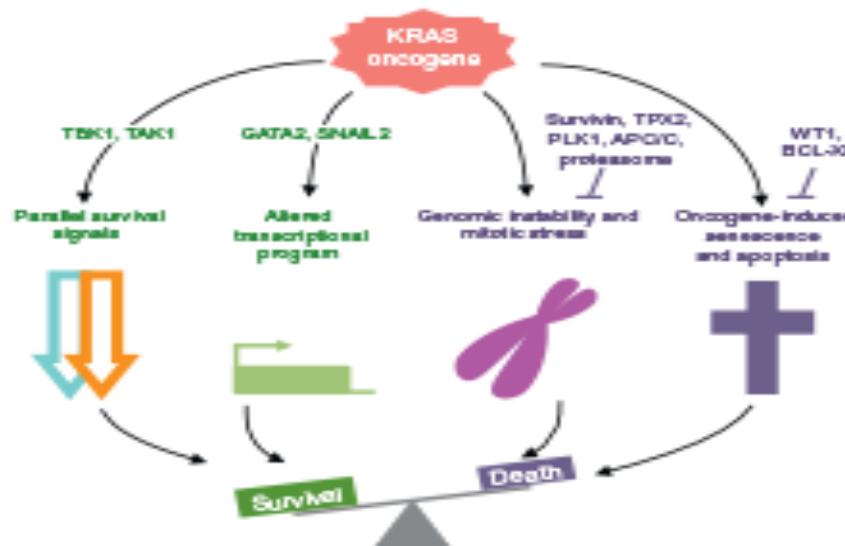
Gene A	Gene B	Viability
WT	WT	✓
WT	Loss	✓
Mutant	WT	✓
Mutant	Loss	✗

Oncogene addiction



Synthetic lethal interactions

Synthetic Lethal Interactions in KRAS Mutant Cells



- Tissue- and genetic context-driven synthetic lethal interactions
- Cooperate with KRAS oncogenic signaling pathways

(Hu & Luo, *The Enzymes* 2014)

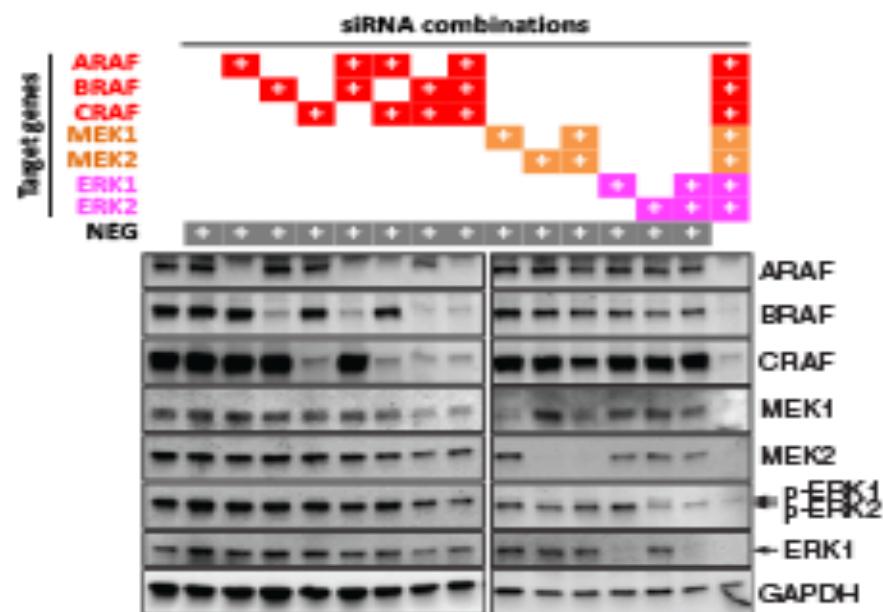
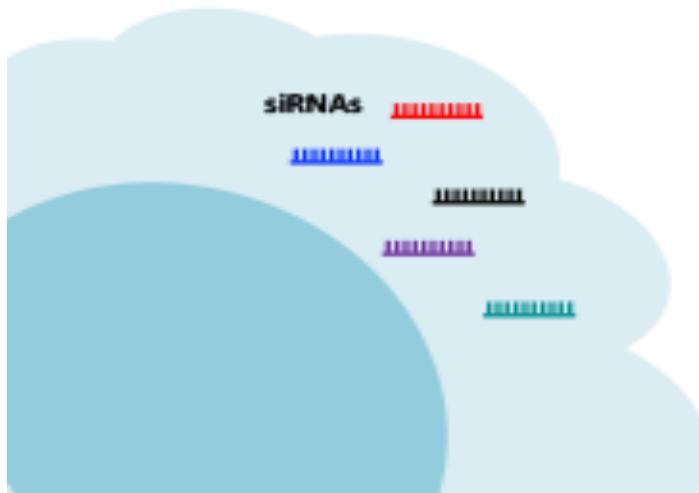
Dissecting the contribution

Dissecting The Contribution of Oncogene and Non-oncogene Addiction in KRAS Mutant Cancer

- What are the critical onco-effectors for mutant KRAS?
 - Distinguishing oncogenic and physiological Ras signaling
 - Critical for mutant KRAS signaling, dispensable in normal cells
- How is KRAS addiction communicated through its effector network?
 - Partitioning of KRAS dependency among pathways
 - Interaction and cooperation among pathways
- What are the critical stress-response pathways in KRAS mutant cells
 - Activated by oncogenic stress, dispensable in normal cells
- What are the rational target combinations downstream of mutant KRAS
 - Genotype selectivity
 - Orthogonal mechanisms of action

siRNA platform

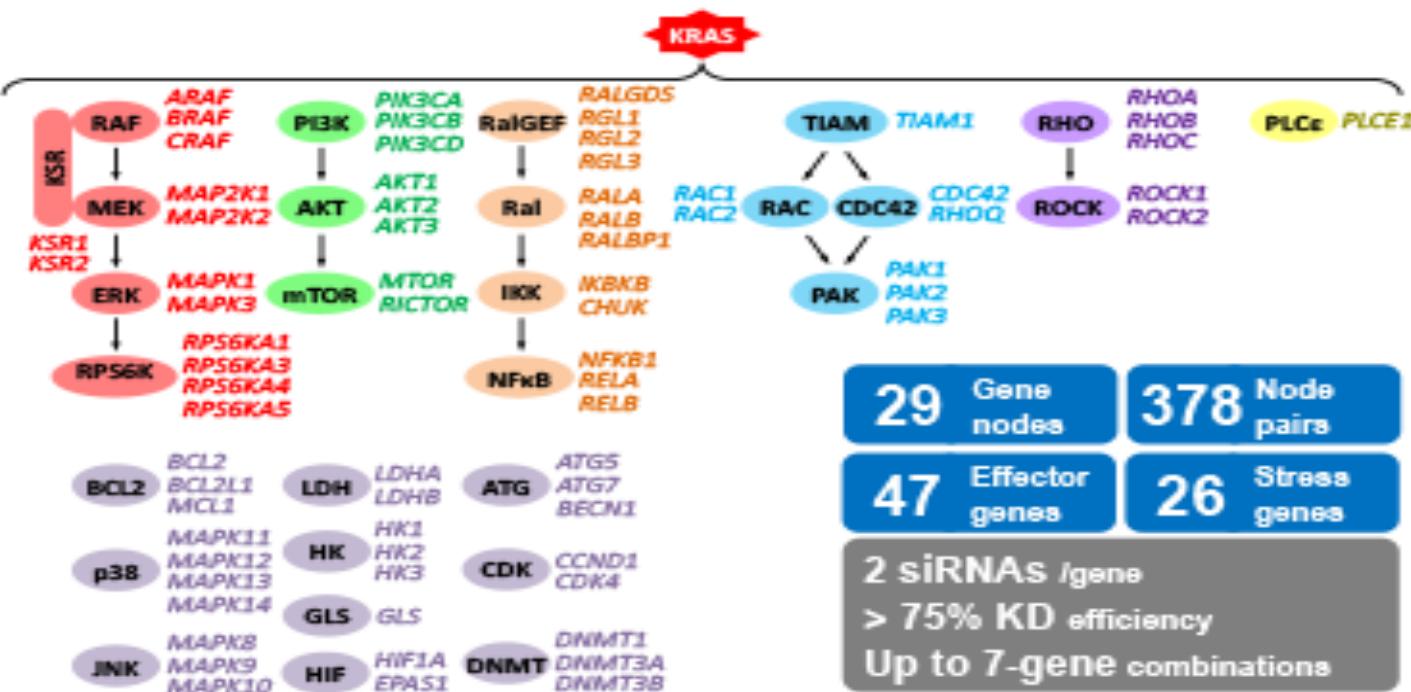
A Combinatorial siRNA Platform to Co-targeting Multiple Genes And Evaluate Target Combinations



(Yuan et al. Cancer Discovery 2014)

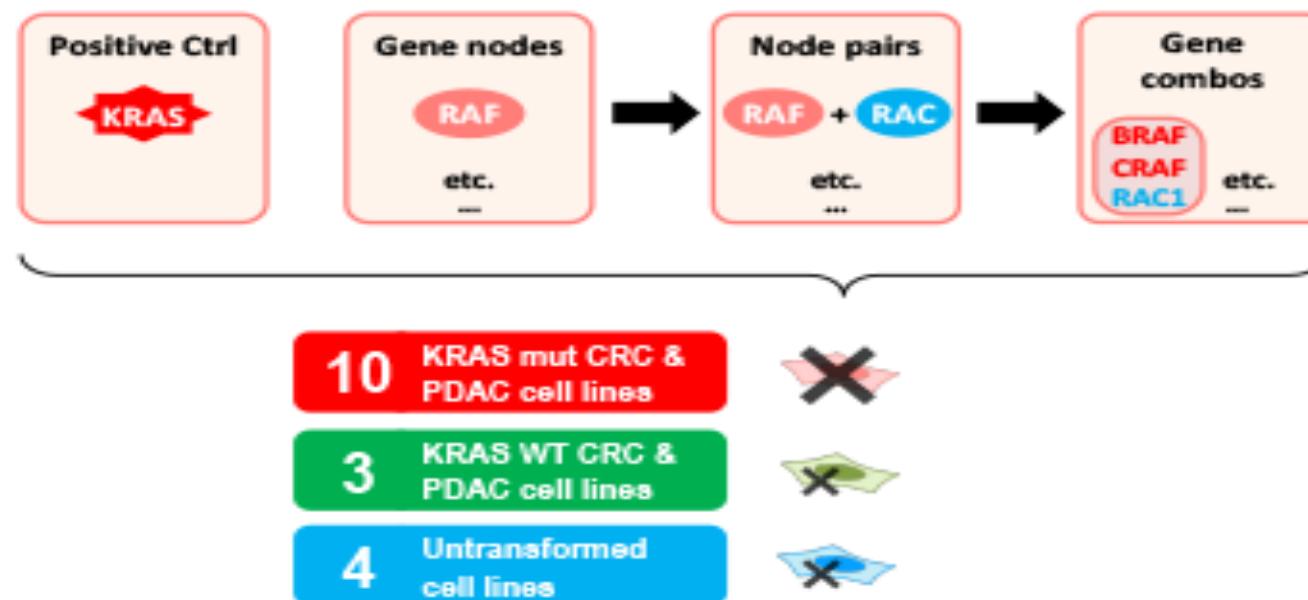
KRAS addiction

Mechanism of KRAS Addiction Through Ras Effector and Stress Response Pathways



Ras effector and stress response

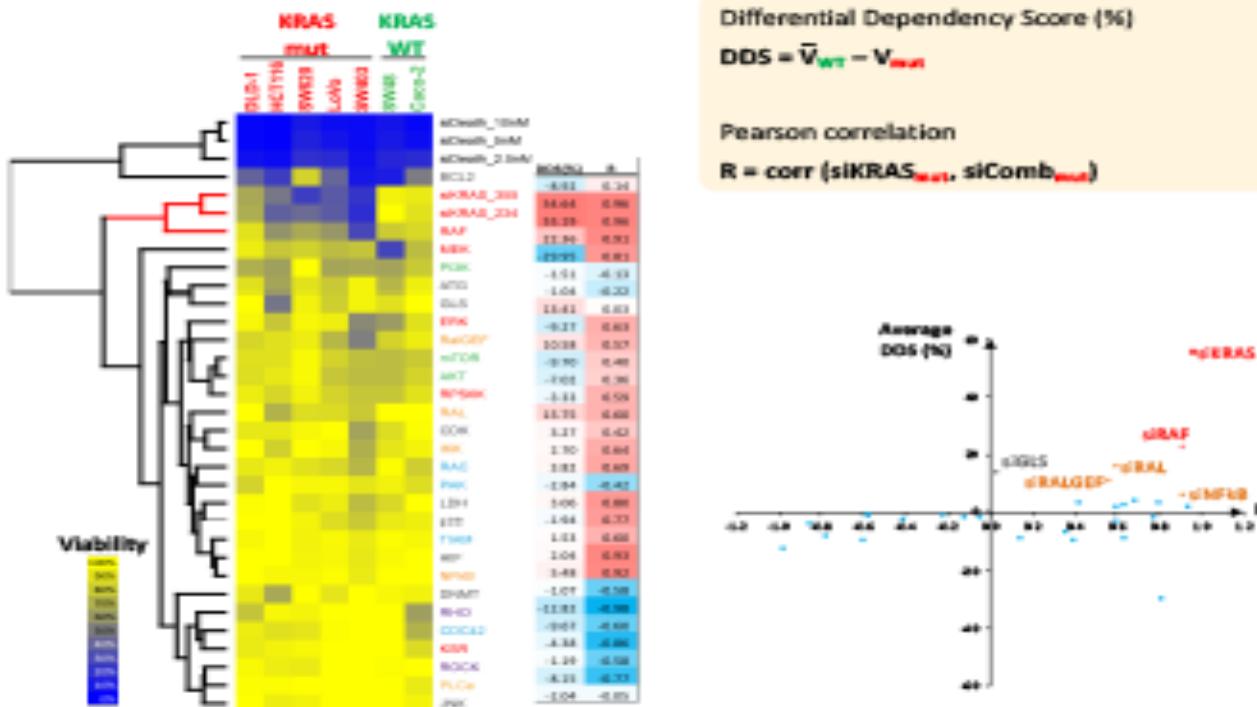
Mechanism of KRAS Addiction Through Ras Effector and Stress Response Pathways



(Lee et al. PNAS 2019)

Single node dependency

Single Node Dependency Profile in KRAS Mutant Cells



(Lee et al. PNAS 2019)

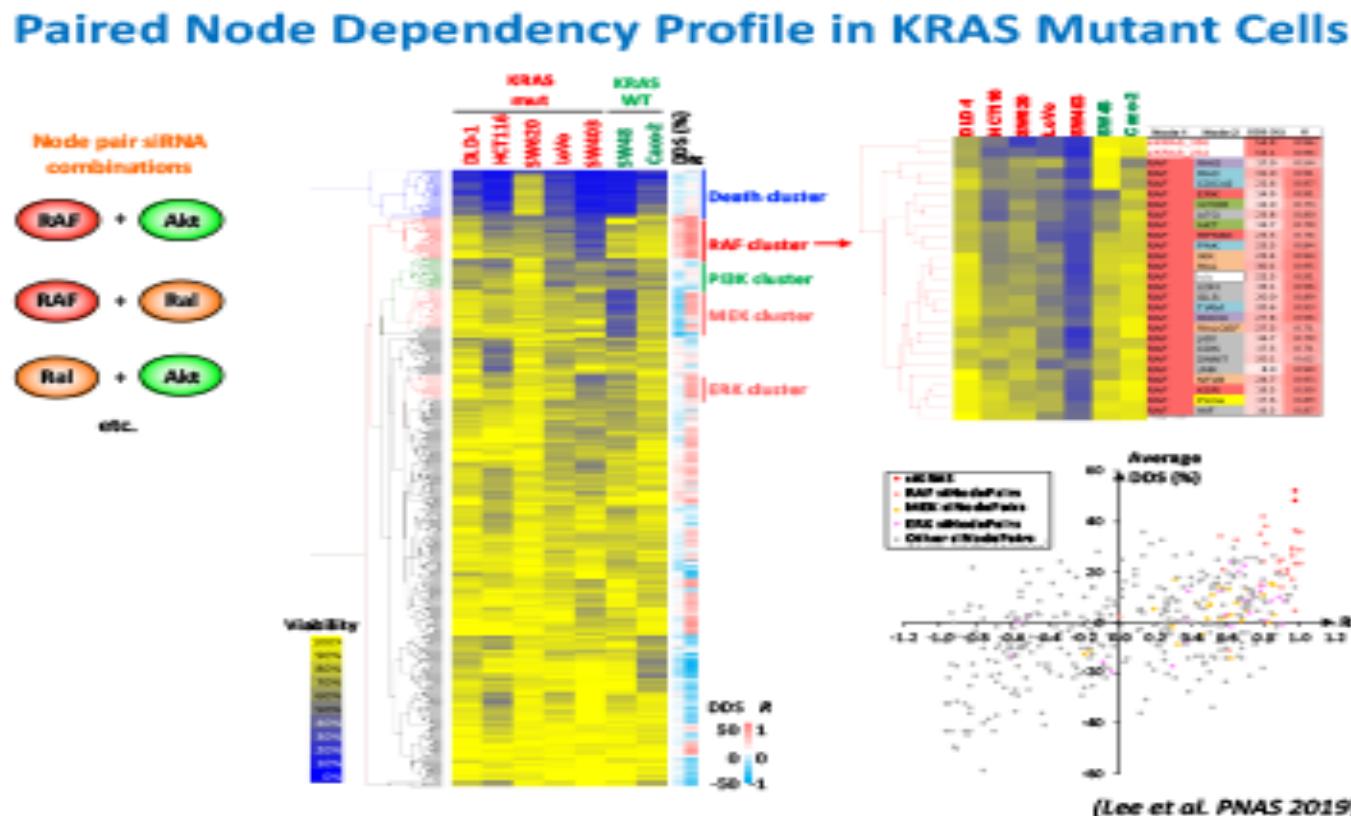
Private node dependency

Private Node Dependency Reveals Heterogeneity in Pathway Utilization for Supporting KRAS Addiction



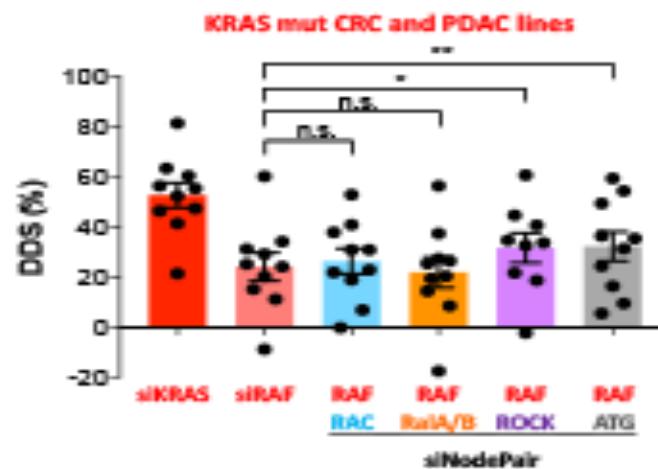
(Lee et al. PNAS 2019)

Paired node dependency



Paired node pair dependencies

Public Node Pair Dependencies in KRAS Mutant Cell Lines



(Lee et al. PNAS 2019)

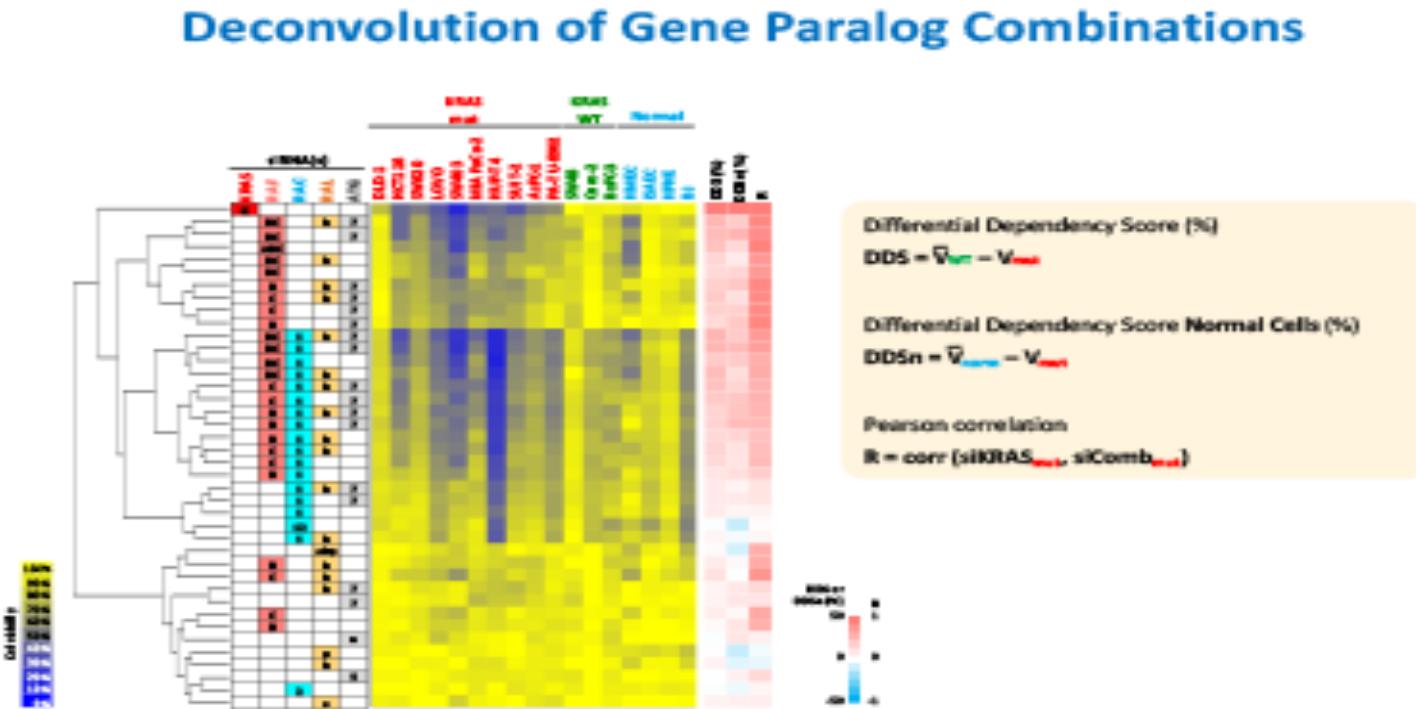
Private node pair dependency

Private Node Pair Dependency Is Dictated by Private Single Node Dependency



(Lee et al. PNAS 2019)

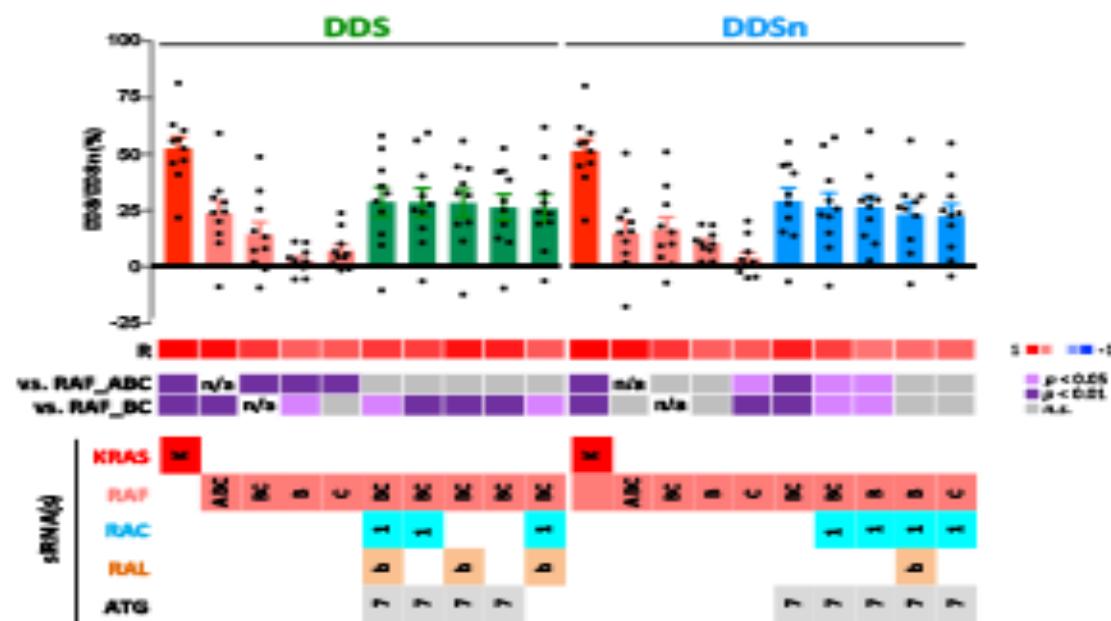
Gene paralog combinations



(Lee et al. PNAS 2019)

Deconvolution

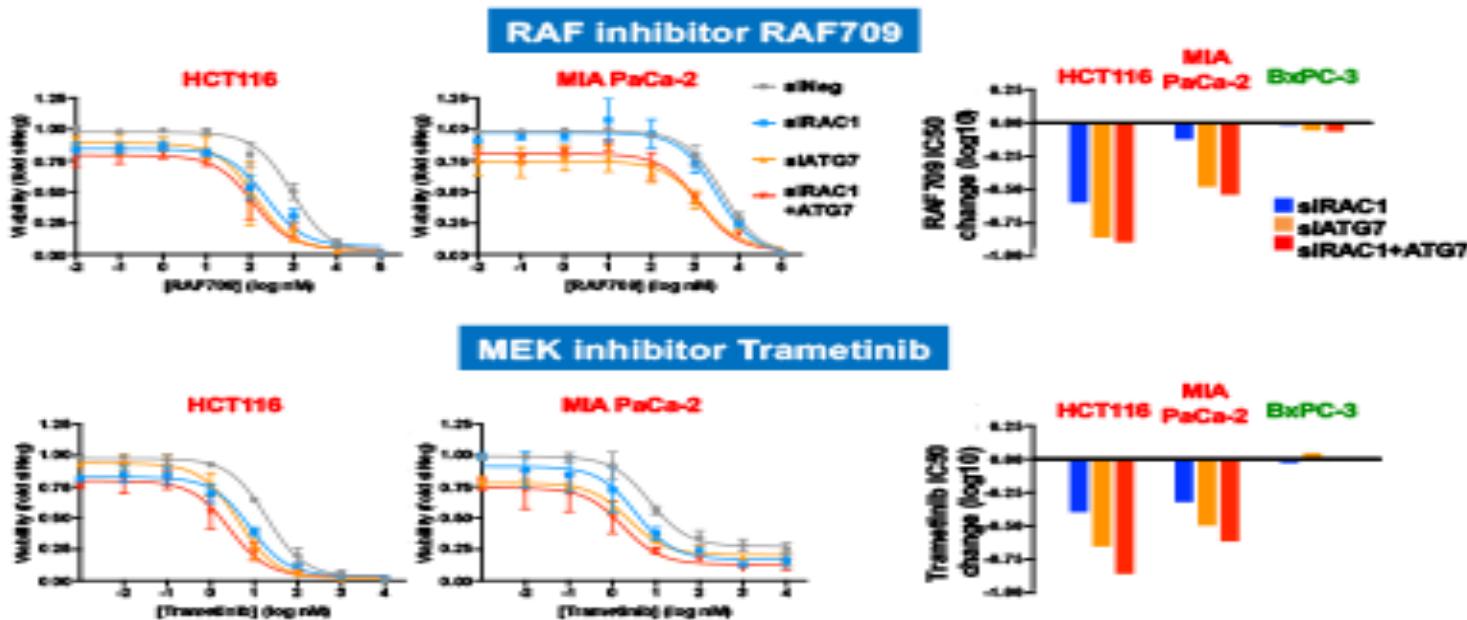
Deconvolution of Gene Paralog Combinations



(Lee et al. PNAS 2019)

Knockdown

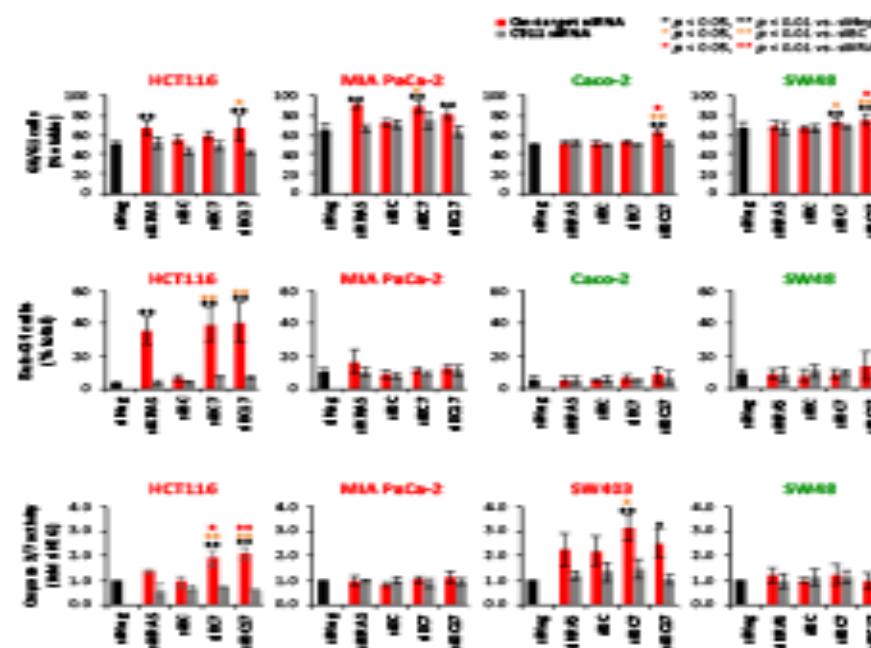
RAC1 and ATG7 Knockdown Sensitizes KRAS Mutant Cells Towards MAPK Pathway Inhibitors



(Lee et al. PNAS 2019)

Cell cycle arrest

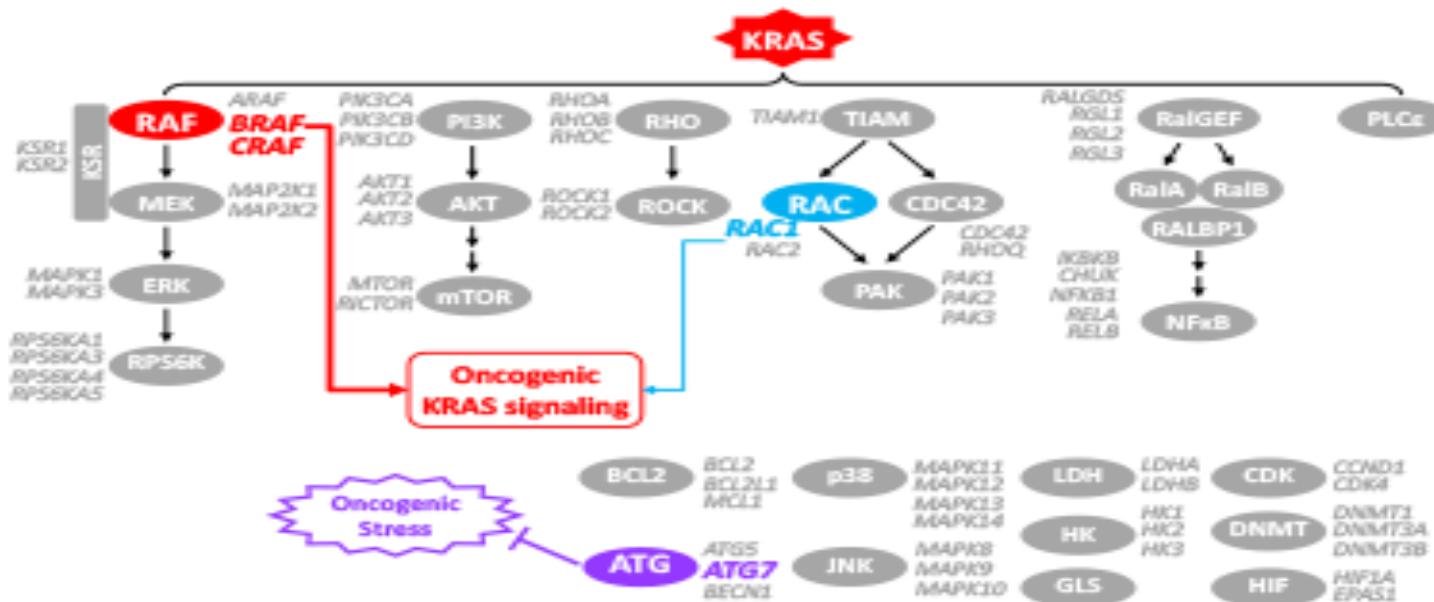
BRAF, CRAF and ATG7 Co-depletion Enhances Cell Cycle arrest and Cell death in KRAS Mutant Cells



(Lee et al. PNAS 2019)

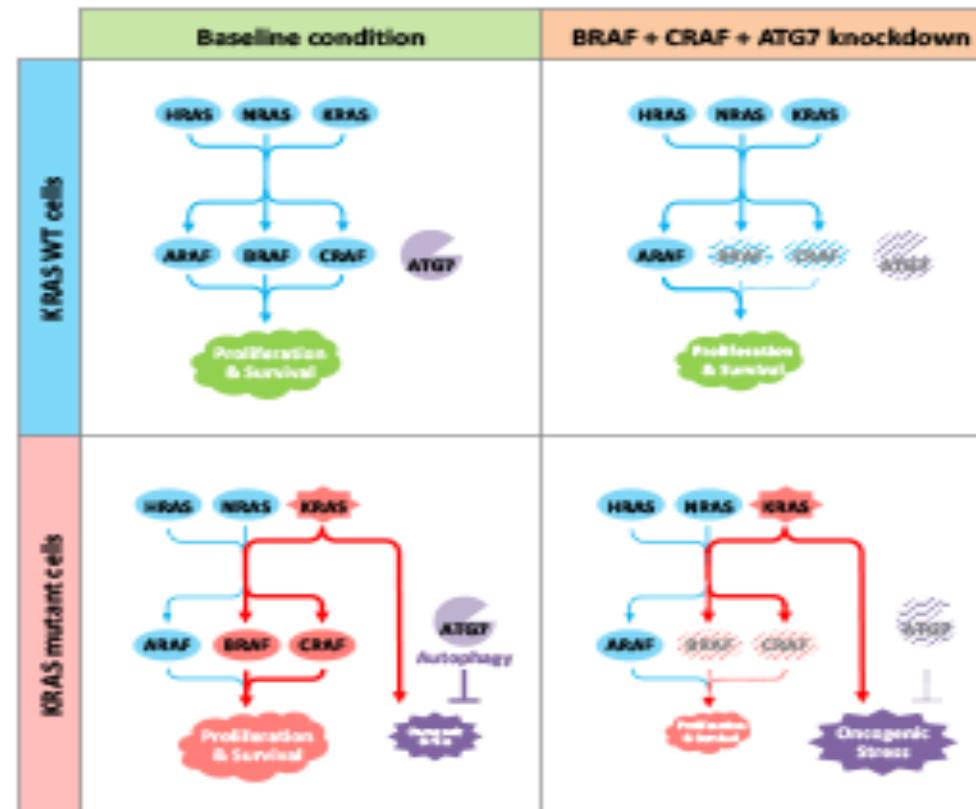
Autophagy pathways

RAF, RAC & Autophagy Pathways Are Critical Mediators of KRAS Oncogene Addiction



RAF kinase and autophagy

RAF Kinases and Autophagy Cooperate to Support KRAS Addiction



Co-targeting

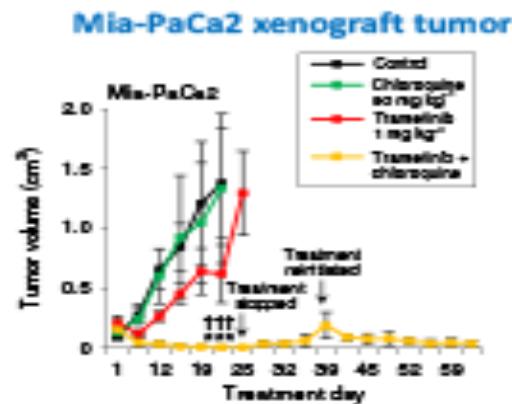
Co-targeting the MAPK and Autophagy Pathway In KRAS Mutant Pancreatic Cancer Cells

Kinsey ... McMahon (*Nature Medicine* 2019)

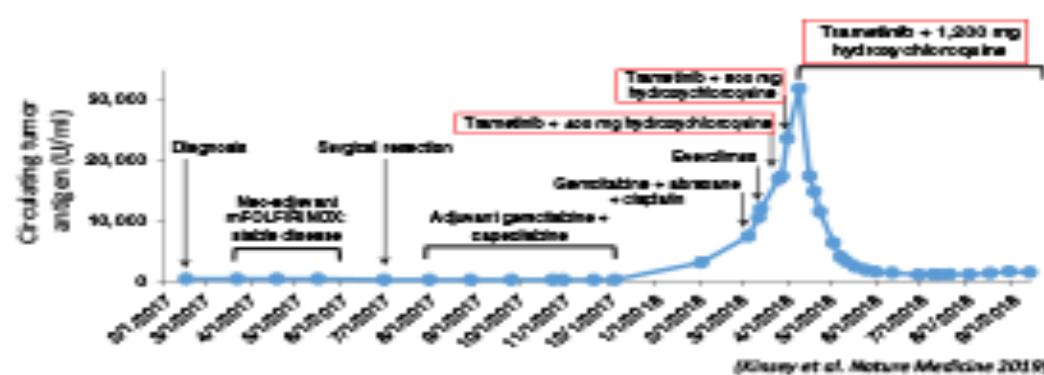
Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers

Bryant ... Der, (*Nature Medicine* 2019)

Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer



KRAS mutant pancreatic cancer patient



Phase I Trial: Trametinib and Hydroxychloroquine in Treating Patients With Pancreatic Cancer

MAPK and autophagy

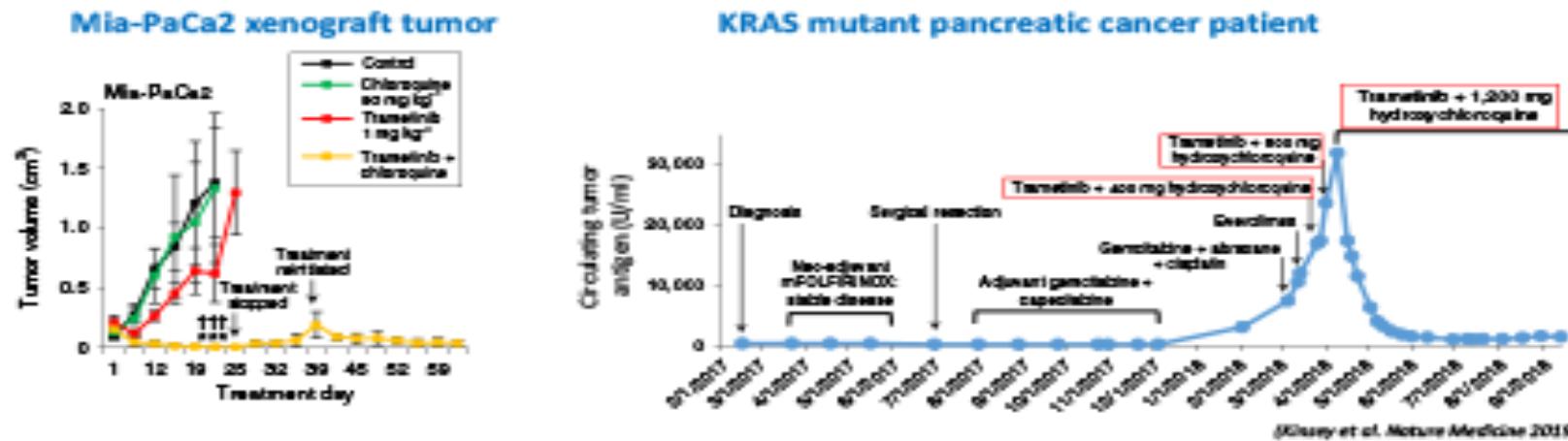
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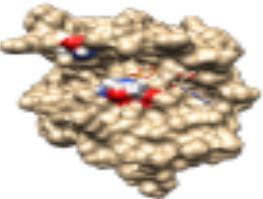
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Acknowledgment

Acknowledgement



Luo Lab		Funding	
Current	Alumni		
Chih-Shia Lee Sean Lin Haibo Zheng Fehad Khan	Qunying Li Meng-tzu Weng Liam C. Lee Dennis Hsu Bing Yu Valentin Giroux	Jordan Smith Abigail Read Joseph Carver Garmen Yuen Xiang Wang	 NATIONAL CANCER INSTITUTE Center for Cancer Research
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